a transmembrane domain (TMD) with an intracellular carboxyl terminus, a poly(asparagine-glycine) linker, and the ligand sequence at the amino terminus. The ability of these tethered peptides to function efficiently provides the experimenter with an exceptional new tool to investigate peptide GPCR physiology both *in vitro* [18] and *in vivo* [2] without having to provision a source of the secreted ligand.

Hyun *et al.* [5] originally showed that the behavioral phenotype of *Pdf receptor* mutant flies (comparable to that of *pdf* mutants) could be rescued by restricting PDF receptor expression to cells that express the clock gene *period*. They concluded that PDF receptor expression restricted to the pacemaker network was sufficient to support its behavioral functions. Hence Choi *et al.* [2] expressed tethered PDF expression in their new studies with similar clock gene promoters. With respect to the original question about the timing of PDF release by pacemakers in the fly brain, they now report that, comparable to over-expressing the normal secreted form of PDF [4], expressing a tethered form of the PDF ligand broadly in clock neurons very potently disrupts normal timing signals. Remarkably, even in a fly mutant for *Pdf*, expression of the tethered PDF can produce rhythmic (albeit abnormally-rhythmic) behavior.

Choi *et al.* [2] argue that their results indicate gated PDF release is not required for the neuropeptide to support gated rhythmic outputs by the pacemaker network. Is tethered PDF a constant activator? In fact, the presentation or clearance of the tethered activator could involve subtle diurnal variation. Likewise PDF receptor signaling within pacemaker neurons may involve downstream elements that exhibit diurnal variation. Without proof of such variation, however, it remains open to conjecture. These compelling new observations on PDF signaling remind us that ingenious technical innovations can inform an old problem with new insight. By the light of a new experimental dawn, even safe conclusions about fundamental circadian mechanisms are subject to serious reconsideration.

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Escape Behaviour: Reciprocal Inhibition Ensures Effective Escape Trajectory

When a zebrafish makes a fast escape response, Mauthner cells directly activate contralateral spinal interneurons which feed reciprocal inhibition to motorneurons on the stimulated side. Ablation of these interneurons in transgenic animals impairs escape responses, indicating their crucial role in survival.

Keith T. Sillar

When it comes to the design of neural circuits controlling escape behaviour, the devil lies in the detail, because even fractions of milliseconds in time or millimetres in distance may mean the difference between survival or being someone's dinner. A new paper by Satou *et al.* [1] provides an elegant demonstration of how a relatively small population of inhibitory spinal interneurons confers a significant selective advantage on the escape performance of larval zebrafish. The paper extends recent research [2] showing that this population of commissural local (CoLo) interneurons is used exclusively during fast escapes and not during other motor behaviours.

For most animals, it's a dog eat dog (or fish eat fish) world out there, and in

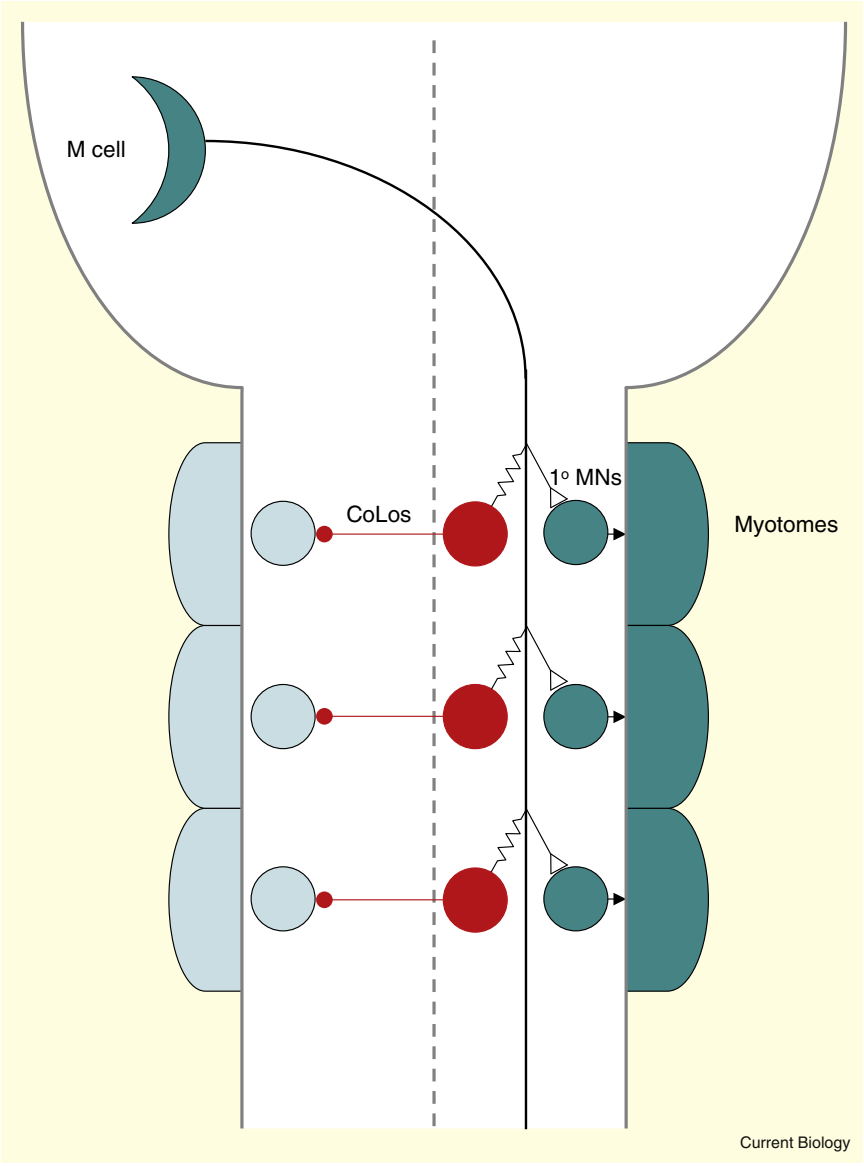

Figure 1. Schematic diagram illustrating position of CoLo interneurons in circuitry of M-cell mediated escape in zebrafish.

The M cell of the brainstem on the stimulated side (aquamarine) activates contralateral primary motorneurons (1° MNs) via chemical excitatory synapses (open triangles) and CoLos (red) via electrical synapses (resistor symbols). CoLos inhibit 1° MNs on the stimulated (pale blue circles) side via chemical connections (small red circles) to ensure ipsilateral myotomes (blue rectangular shapes) are silenced when the contralateral motor system (aquamarine structures) is activated. The mideline is shown as a dashed line.

order to escape effectively speed is of the absolute essence. In fish (and also amphibian tadpoles), rapid responses to alarming stimuli, such as vibrations in the water column, are effected by Mauthner (M) cells, a pair of conspicuously large, fast conducting reticulospinal interneurons with somata located on either side of the brainstem (Figure 1) [3,4]. The M cell circuitry is responsible for triggering 'C-start' escape behaviour in which the body bends rapidly into a C shape, orienting the organism away from the threatening stimulus, before darting off in the opposite direction ([3–5]; see [6] for a Quick guide on M cells).

Much of the basic wiring diagram has been known for a long time: the M cell on the side ipsilateral to the stimulus receives sensory inputs primarily, though not exclusively, from auditory hair cell afferents via synapses called club endings, which have both electrical and chemical components. These bring the M cell rapidly to threshold and, once this is breached, a single action potential is usually triggered that propagates along the M cell axon. Within the brainstem the M cell axon crosses to descend in the contralateral spinal cord (Figure 1). In each spinal segment the M cell excites large primary motorneurons which in turn activate the segmented, fast twitch myotomal muscles, which are the effectors of the C-start. So far, so good; we have a relatively simple tri-synaptic pathway containing large, fast conducting neurons with the incorporation of electrical synapses at key stages to enhance speed of transmission.

A key feature of successful escape is that when one side of the fish is fully activated to produce the initial C-bend, the other side must be prevented from contracting to ensure the intended escape movement is not counteracted. It has been known for over 40 years that spinal motorneurons on the stimulated side are inhibited almost simultaneously with the activation of the contralateral motor pool [7]. But although this suggested the existence of commissural interneurons to mediate crossed inhibition, and some features of these interneurons were described physiologically and anatomically [8,9], they were only recently identified in zebrafish as the CoLo interneurons [1,2]. The precise timing of the inhibition pointed to a population of interneurons activated by the M cell via electrical synapses but direct evidence was lacking.

In the modern era of neuroethology the zebrafish has come to the fore as a model system which offers opportunities to answer hitherto intractable problems such as what role do CoLos play during rapid escapes, the main question addressed in the paper by Satou *et al.* [1]. The first hurdle was to visualize the interneurons under investigation and this was achieved using an ingenious enhancer trap line of zebrafish in which the CoLos express green fluorescent protein (GFP). It turns out to be a very small population of interneurons, at least in the young larvae studied in this paper [1], with only one CoLo per hemi-segment (Figure 1). Apart from being helpful in characterising the CoLo interneuron population, the GFP tag greatly facilitates studying the individual

morphology of CoLos following electroporation with rhodamine dye and their physiology using visually guided patch clamp recordings.

The notion that CoLos are involved in C starts [2] was confirmed by showing that in α-bungarotoxin paralysed larvae, they are active at the onset of escape sequences discharging a brief burst of action potentials. When paired recordings were made from the M cell on one side and a CoLo on the opposite side monosynaptic excitatory connections were revealed with a short and constant synaptic delay. The connections had a high fidelity, faithfully following the M cell when it is made to fire at 100 Hz, consistent with electrical connections, and CoLo responses to M cell spikes were abolished by the gap junction blocker carbenoxolone. In summary, M cells on one side excite CoLos on the opposite side via electrical connections and therefore effectively inhibit ipsilateral primary motorneurons at the same time as they excite contralateral primary motorneurons via chemical excitatory connections.

This wiring diagram ensures that at the very moment when the contralateral motor pool is switched on, the ipsilateral motor pool is switched off by virtue of the CoLos crossed chemical inhibitory output synapses. In effect, the presence of electrical connections between M cells and contralateral CoLos allows the trigger neurons to mediate simultaneous chemical excitation on one side and chemical inhibition on the other. This should, in theory at least, ensure that in the event of the other M cell being inadvertently fired, the CoLos will negate its effects and prevent a counteracting muscle contraction which might impede escape. But how can this be tested? How can the gap between circuits and behaviour be bridged?

The enhancer trap line of zebrafish, with its GFP-tagged CoLo interneurons, provides an excellent opportunity to observe escape behaviour in the presence and the absence of the CoLos, because they can be laser-ablated. In this way escape can be triggered by a sound/vibratory stimulus and the escape trajectory of control fish compared with animals in which a sub-population of the CoLos had been deleted from the network. Normally these stimuli trigger escape responses at an incredibly short latency with the first movement detectable a mere 2 ms after the stimulus; human reaction times are orders of magnitude slower at around 200 ms on average!

Using a range of CoLo ablation paradigms such as unilateral or bilateral, rostral or caudal, Satou *et al.* [1] provide convincing evidence that CoLos prevent co-activation of the muscles on the two sides, consistent with the idea that the stimuli used in this study on larval zebrafish often activate both M-cells with a short delay, and the CoLos negate the effects of the second M-cell in the sequence. Any reduction in the potency of the CoLo population led to impaired escapes in which the full C-shape was not accomplished because the ipsilateral myotomes in segments lacking CoLo input were able to contract. Calcium imaging experiments confirmed that the M-cells were indeed often co-activated, supporting a crucial role for CoLos in turning off counterproductive motor commands.

Almost since the dawn of the field of neuroethology there has been a frustrating gulf between knowledge about cells, synapses and circuits, and an understanding of their precise roles in particular patterns of behaviour. Even a complete circuit diagram is now recognised as being insufficient to explain the fine detail of a given action or the inherent flexibility of even the simplest behaviours like reflexes or locomotion. All too often authors have laid claim to a complete understanding of this circuit or a full description of that behaviour. But we can only describe what we visualise or record from, remaining blissfully ignorant of circuit details which we are unaware of, but which may be crucial for an overlooked aspect of the behaviour under investigation. The recently discovered zebrafish CoLos are a good example. Like the M cells that drive them, they are integral components of a reflex circuit designed to produce rapid escape responses which propel the fish in the opposite direction to a threatening stimulus. The CoLos appear to play no role in other motor behaviours like struggling or swimming and indeed they are actively inhibited during the swimming that usually follows M cell activation. These recent studies by Satou *et al.* [1] and Liao and Fetcho [2] are elegant examples of the way in which modern analytical approaches to the genetically tractable zebrafish model system are finally allowing the gap between neural circuits and behaviour to be bridged.

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